CHARACTERIZATION OF VALUE ADDED FOOD PRODUCTS FROM UNDERUTILIZED PULSES AND BIO-FORTIFIED WHEAT

SYNOPSIS

Submitted to Eternal University

In the partial fulfilment

For the degree of

Doctor of Philosophy in Food Technology

By

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February, 2020

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1. TITLE: Characterization of value added food products from bio-fortified wheat and underutilized pulses

2. INTRODUCTION

Wheat (*Triticum aestivum*) is the key staple food consumed worldwide and its demand is rising. Micronutrient deficiency termed as micro-nutrient malnutrition is affecting more than 40% population of the world, particularly in several developing nations. More than 3 billion people worldwide suffer from the deficiency of micronutrients (Liu et al., 2006). A major part of the population in developing countries mainly women and children are greatly influenced by the scarcity of key micronutrients such as iodine (I), zinc (Zn), iron (Fe), vitamin A and selenium (Se) (Ghandilyan et al., 2006). This malnutrition influences health of individual people; enhance the healthcare cost of society, and decreases economic productivity (Welch and Graham, 2004). Conventional efforts are made to resolve the problems of malnutrition caused due to deficiency of micronutrients and have focused on fortification of micronutrients in diet of people (Ghandilyan et al., 2006). Moreover, these techniques are not sustainable, mainly in countries where fish and animal products having immense key micronutrients are not afforded. Hence, cereals as their staple food that do not provide a huge quantity of micronutrients are consumed here is unable to meet the nutritional requirements of humans (Shi et al., 2008).

In current years, a mineral deficiency solution known as ‘‘biofortification’’ has been used (Uauy et al., 2006). Genotypes are developed by wheat breeders for Zn biofortification mainly under Harvest Plus program of Consultative Group on International Agricultural Research. Moreover, the nutrient content of grains is negatively linked with the yield of grain. High hunger risk is faced by developing countries and thus cannot negotiate on grain yield for biofortification of Zn (Xu et al., 2011).

The density of minerals and vitamins in a crop through transgenic techniques, plant breeding, and agronomic practices are increased by the process of biofortification. Staple biofortified crops when utilized regularly will give measurable improvements in human nutrition and health (Bouis and Saltzman, 2017). These biofortified staple foods with selected micronutrients particularly for public health significance are known as a strategy and this approach has the capacity to improve global welfare at fairly low cost and it can be of particular advantage to poor people of rural populations (Jorge et al., 2009).

Dietary deficiency of essential micronutrients like iron (Fe) and zinc (Zn) influences more than two billion people worldwide (White and Broadley, 2009). Malnutrition of key micro nutrients is a major problem than low energy intake as well as poor dietary quality is faced in several parts of world (Stewart et al., 2010) and under-five about 20% of children deaths takes place due to Zn, Fe, vitamin A, and iodine insufficiency (Prentice et al., 2008). Several countries having huge deficiencies of micronutrients consume cereal-based food that signifies the main amount of the daily diet (Bouis, 2007; Cakmak, 2008). Developing countries are now working to overcome these mineral deficiencies by the biofortification process of staple crops having vitamins and essential minerals; it is the approach that is considered as the most economical resolution to human micronutrient malnutrition (Cakmak, 2008; Bouis, 2007; Peleg et al., 2009; Welch and Graham, 2004).

Pulses are also recognized as low GI (glycemic index) foods. Pulses like Pigeon peas, lima beans, horse gram, etc. helps to overcome malnutrition of minerals and other micronutrients. Pulses have various components to increase GI such as high dietary fiber content and low release of carbohydrates. In diabetes mellitus a study by (Dilawari et al., 1987) reported anti-diabetic effect of pulses on the level of sugar in blood and recommended that consumption of pulses could help to control glucose level in blood of diabetic patients. Venn and Mann (2004) found that pulses are helpful in management and prevention of individuals diabetes and great evidences are presented for whole-grain foods like pulses. The main cause of several ailments i.e. Malnutrition exists quickly in various developing countries. To overcome the deficiency, various pulses like pigeon pea, horse gram, lima bean, adzuki bean, etc. can play an important role in a poor man’s diet particularly the deficiency of protein. However, fermented pigeon pea plays also a great role where certain nutrient bioavailability is further enhanced, and when this fermented flour of pigeon pea is added to pasta it improves its true protein digestibility and PER by 6% and 72%, respectively (Torres

et al., 2006). It is exceptional among the pulses that they exhibit great tolerance against environmental stresses, optimal nutritional profiles, provides elevated-biomass productivity, and provides nutrients and moisture to the soil (Odeny, 2007).

Further pulses contain lysine in a vast amount, which is deficient in cereals. Whereas, cereals are rich source of methionine which is considered to be deficient amino acid in pulses. Therefore, the combined use of pulses and biofortified (Fe and Zn) wheat for the development of functional foods can be helpful for the alleviation of deficiency of these essential amino acids as well as the essential micronutrients like iron and zinc. Further, the use of germination treatment can increase the nutritive quality by decreasing anti-nutritional components and increasing bioavailability.

Objectives:

Keeping in view the nutritional and health benefits of biofortified wheat and underutilized pulses, the current study is undertaken with the following objectives:

1. To study the physicochemical analysis of underutilized pulses such as adzuki bean (*Vigna angularis*), horse gram (*Macrotyloma uniflorum*), pigeon pea (*Cajanus cajan*), Lima beans (*Phaseolus lunatus*), and biofortified wheat (Fe and Zn biofortified).
2. Effect of germination on the nutritive value and anti-nutritional components of underutilized pulses and biofortified wheat
3. To find out in-vitro protein digestibility and bioavailability of micronutrients (iron and zinc) in raw and treated grains
4. To optimize/standardize food products by incorporating varying levels of germinated grains on the basis of sensory evaluation
5. Evaluation of physicochemical parameters, functional and textural characteristics of standardized food product
6. To evaluate the shelf-life of developed functional food products

Knowledge gap

As the lysine which is an essential amino acid is lacking in cereals but it exist in plenty in pulses, on the other hand, methionine another essential amino acids is present in abundance in staple cereals especially wheat, hence combined use of wheat and pulses will balance the the content of amino acids deficient in either of them. Further, the use of germination will improve the functional as well as nutritional characteristics of grains. Therefore, the

combined use of Fe and Zn biofortified wheat and underutilized pulses and their commercialization will help to alleviate the deficiency of Fe and Zn as well as protein-energy malnutrition (PEM) by providing the proper amount of proteins and a balanced proportion of essential amino acids.

3. EXPECTED NEW KNOWLEDGE

The application of germination will improve the nutritional and functional characteristics of pulses and wheat by reducing the anti-nutritional components. Reduced anti-nutritional factors will help to enhance the bioavailability of nutrients in developed food products. This study will help to develop the scope for better utilization of these underutilized pulses and biofortified wheat in the development of functional foods with balanced proportion of amino acids and will also encourage industries to fortify food formulations and launching of functional foods based on these ingredients.

4. REVIEW OF LITERATURE

Pulses are considered among the most vital categories of food and are widely consumed to cover essential energy and protein needs. In addition to their high dietary fiber and low lipid content promising evidence stresses the significance of pulses as a carrier of various components of potential biological significance, such as enzyme inhibitors, phytates, lectins, phytosterols, oxalates, saponins, polyphenols, etc (Dilis and Trichopoulou, 2009). Pulses are loaded with tremendous amounts of dietary fiber, protein, phytochemicals, and micronutrients (Messina, 1999). It supplies various bioactive components in fewer amounts in addition to their nutrients with considerable physiological and/or metabolic effects. These bioactive components are termed as the non-nutrient bioactive components viz. high molecular tannins, flavonoids, and phenolic acids (Siddhuraju and Becker, 2007). These components have great nutraceutical and medicinal characteristics and have an inhibitory role in decreasing several ailments such as diabetes, obesity, and cardiovascular diseases (Bazzano et al., 2001).

Azra et al. (2008) has studied that kidney bean (*Phaseolus vulgaris*) are rich in lysine and sulfur-containing amino acids, and nutritional value get increased in a balanced mixture of grains than alone ingredients (Kadam and Salunkhe, 1985). The effect of various processing techniques like soaking in solutions of sodium bicarbonate or citric acid, germination, soaking, and cooking on anti-nutritional factors (total polyphenols, phytic acid, hydrocyanic acid, and tannins) in red kidney bean decreased their anti-nutritional factors.For

example 25% of cyanide contents were decreased efficiently by the process of cooking after soaking them in sodium bicarbonate solution. When grains were cooked after soaking in citric acid or sodium bicarbonate solutions, it removed almost all tannin contents from grains.

Rocio et al. (2010) studied that pulses have a great number of bioactive components like lectins, enzyme inhibitors, oligosaccharides, phytates, phenolic components, etc. Lectins decrease nutrient absorption and enzyme inhibitors can reduce protein digestibility, but after cooking this effect was significantly reduced. Phytic acid has an antioxidant activity that prevents the damage of DNA. Phenolic components have antioxidant and other significant biological and physiological characteristics, and galactooligosaccharides possess prebiotic activity. These components have both the overlapping as well as complementary mechanisms of action, like the stimulation of the immune system, antioxidant, modulation of detoxifying enzymes, regulation of hormone and lipid metabolism, anti mutagen and antiangiogenic effects, tumour reduction, and induction and promotion of apoptosis. Secondary metabolites having health benefits are currently termed as nutraceuticals and functional food ingredients.

Prasad and Singh (2015) studied that horse gram an underused pulse crop can be grown in adverse climatic conditions. Besides its various nutritional importance, it is consumed to diminish the risk of several ailments because of non-nutritive bioactive components in horse gram. These bioactive components like phenolic acid, phytic acid, and proteinase or enzymatic inhibitors have great physiological or metabolic effects. It gains its importance because it is used to treat urinary diseases, kidney stones, common cold, piles, fever, and throat infection. It reduces the risk of diabetes, intestinal diseases, dental caries, cardiovascular disease etc.

The Phytate content of pulses can be significantly reduced by use of various processing treatments The biological processing techniques like soaking cooking, fermentation, germination, and hydrothermal processing decreased the phytic acid by 75%, 50-60%, 80%, 50-70%, 95% respectively (Sandberg et al., 1991). Furthermore, the phytic acid is sensitive to heat as it got inactivated during longer exposure to high temperature. (Shamsuddin and Vucenik, 2005). Mubarak (2005) investigated that phytic acid content of mungbean pulses get reduced to 28.7% and 30% by germination. Futher it was found to get reduced by 90-95% by fermentation process due to the production of lactic acid and citric acid, which support the activity of enzyme phytase resulting in reduction of phytate content (Gibson et al., 2006).

Kumar et al. (2010) found that de-phosphorylation of phytate present in foods occurs by enhancing the naturally occurring phytate degrading enzyme activity in seeds. During germination, a pulse seed utilizes this phytate as a rich source of inorganic phosphate and tends to enhance the nutritive value and palatability of these pulses (Kumar et al., 2010). Luo et al. (2013) studied the effect of processing treatments like fermentation and germination on phytic acid contents and found that fermentation reduced the phytic acid (72%) followed by germination (45%). Adeniran et al. (2013) found reduction in phytate content of 77.82 and 73.53% for locust and lima bean seeds, respectively after fermentation. It has been demonstrated in many studies that significant phytate reduction also takes place during the germination process because of the same reason. Vitamins and mineral contents of biofortified staple foods are not delivered equally per day as compared to the fortified foods or supplement products, but they, in turn, enhance the intake of micronutrients (Bouis et al., 2011).

5. TECHNICAL PROGRAMME OF WORK

Experiments planned in accordance with the objectives are given under the following subheads

Objective: 1

Name of Experiment: To study the physicochemical analysis of underutilized pulses such as adzuki bean (*Vigna angularis*), horse gram (*Macrotyloma uniflorum*), pigeon pea (*Cajanus cajan*), Lima beans (*Phaseolus lunatus*), and biofortified wheat (Fe and Zn biofortified).
Location: Analytical laboratories of the Department of Food Technology

Methodology: As per standard protocols

a) Observations to be recorded: Physical and functional parameters

i. Thousand-grain weight
ii. Bulk density
iii. Tap density
iv. Dimensional properties (Length, breadth and thickness)
v. Water absorption capacity (WAC)
vi. Water solubility index (WSI)
vii. Oil absorption capacity (OAC)

b) Nutritional, anti-nutritional and functional components

i. Proximate composition

ii. Mineral contents (Ca, Fe, Zn, Mn, Cu)

iii. Anti-nutritional factors (Phytic acid, tannins, Trypsin inhibitor actibity)

iv. Total Phenolic contents

v. Flavonoid contents

vi. Antioxidant activity

c) Optical properties

Colour values (L, a and b values)

Objective 2

Name of Experiment: Effect of germination on nutritive value and anti-nutritional components of underutilized pulses and biofortified wheat

Location: Food analysis and quality control labs of the Department of Food Technology

Methodology: As per standard protocols

Observations to be recorded:

a) Effect of processing treatment (as given below) on physicochemical parameters, anti-nutritional components, and functional properties of raw ingredients will be studied

T1- soaking for 12 hrs and drying in the oven at 50 °C

T2- Germination for 24 hrs and drying in an oven at 50 °C

T3- Germination for 48 hrs and drying in the oven at 50 °C

T4- Germination for 72 hrs and drying in an oven at 50 °C

*Treatments will be screened based on their effectiveness in enhancing the nutritive value and decreasing anti-nutritional component and the best treatment will be utilized for the development of food products.

b) Nutritional, anti-nutritional, and functional components as mentioned in objective 1(b

Objective 3

Name of Experiment: To find out *in-vitro* protein digestibility and bioavailability of micronutrients (iron and zinc) in raw and treated grains

Location: Food analysis and quality control labs of the Department of Food Technology

Methodology: As per standard protocols

Observations to be recorded:

a) Protein digestibility (in vitro)

b) Bioavailability of micronutrients (iron and Zinc)

Objective 4

Name of Experiment: To optimize/standardize food products by incorporating varying levels of germinated grains based on sensory evaluation

Location: Food processing pilot plants and quality control lab of the Department of Food Technology

Methodology: As per standard protocols

Grains treated with best processing treatments (screened based on increasing nutritive value, and decreasing the anti-nutritional components) will be utilized for the preparation of functional food products.

The judicious combinations of processed grains will be used for the preparation of different composite flour formulations to be utilized for the development of functional food products (biscuits, breads, and pasta products) by taking whole wheat flour products as control.

The best formulation for each product will be screened based on organoleptic evaluation of different formulations.

Observations to be recorded: Organoleptic evaluation will be done using the 9-point hedonic scale for colour, flavor, texture, taste, and overall acceptability

Objective 5

Name of Experiment: Evaluation of physicochemical parameters, functional and textural characteristics of standardized food product

Location: Food analysis and quality control labs of Department of Food Technology

Methodology: As per standard protocols

Observations to be recorded:

a) **Nutritional. Anti-nutritional and functional components:** As mentioned in objective 1 (b)

b) Anti-oxidant activity (% radical scavenging activity)

c) **Reducing capacity**

d) **Metal chelating (Fe+2) activity**

e) **Protein digestibility**

f) **Bioavailability of micronutrients (Zn and Fe)**

g) **Textural characteristics:** (Texture profile by texture analyzer)

Objective 6

Name of Experiment: To evaluate the shelf-life of developed functional food products

Location: Food analysis and quality control labs of the Department of Food Technology
Methodology: As per standard protocols

Physico-chemical parameters, organoleptic, and microbiological evaluation of products packed in different packaging materials (HDPE and Laminates) will be assessed at different time intervals using standard protocols.

Observations to be recorded:

a) **Nutritional, anti-nutritional, and functional components:** As mentioned in objective 1 (b)

b) **Organoleptic evaluation by the 9-point hedonic scale**

c) **Total plate count**

Statistical Analysis: By SPSS statistical package using one way/two way ANOVA and test of significance.

6. REFERENCES

Adeniran HA, Farinde EO, and Obatolu VA (2013) Effect of heat treatment and fermentation on anti-nutrients content of Lima Bean (*Phaseolus lunatus*) during Production of daddawa analogue. ***Annu Res Rev Biol***. **3(3)**: 256-266

Azra Yasmin, Aurang Zeb, Abdul Wajid Khalil, Gholam Mohi-ud-Din Paracha and Amal Badshah Khattak Food Bioprocess Technol (2008) Effect of Processing on Anti-nutritional Factors of Red Kidney Bean *(Phaseolus vulgaris*) grains. ***Food Bioprocess Technol.*** **1**:415-419

Amy Saltzman, Howarth E Bouis, Improving nutrition through biofortification: A review of evidence from ***Harvest Plus, 2003*** through 2016.

Bazzano L, He J, Ogden LG, Loria C, Vuputuri S, Myers L (2001) Legume consumption and risk of coronary heart disease in US men and women. ***Arch Intern Med.*** **161**:2573-2578

Bouis, HE (2007) The potential of genetically modified food crops to improve human nutrition in developing countries. ***J Dev Stud.*** **43(1)**:79-96

Bouis, HE Hotz, C Mc Clafferty, B Meenakshi, JV Pfeiffer, WH (2011) Biofortification: A new tool to reduce micronutrient malnutrition. ***Food Nutr Bull.*****32**: 31S-40S

Cakmak, I (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification. ***Plant and soil.*** **302(1-2**): 1-17

Dilawari J, Kumar V, Bhatnagar R, Dash R (1987) Effect of legumes on blood sugar in diabetes mellitus. ***Indian J Med Res.*** **85**:184–187

Ghandilyan A, Vreugdenhil D, Aarts M G M (2006) Progress in the genetic understanding of plant iron and zinc nutrition. ***Physiol Plant.*** **126**: 407-417

Gibson RS, Perlas L and Hotz C (2006) Improving the bioavailability of nutrients in plant foods at the household level***. P Nutr Soc*****.* 65**: 160– 168

Jorge L, Rosado, K Michael Hambidge, Leland V Miller, Olga P Garcia, Jamie Westcott, Karla Gonzalez, Jennifer Conde, Christine Hotz, Wolfgang Pfeiffer, Ivan Ortiz-Monasterio, Nancy F Krebs (2009) The Quantity of Zinc Absorbed from Wheat in Adult Women Is Enhanced by Biofortification. ***Nutr J.*** **139**: 1920–1925

Kadam, S S and Salunkhe, D K (1985) Nutritional composition, processing, and utilization of horse gram and moth bean. ***Crit Rev Food Sci Nutr*****.* 22(1)**: 1-26

Kumar V, Sinha AK, Makkar PSH and Blecker K (2010) Dietary roles of phytate and phytase in human nutrition. ***J Food Chem*****.* 120**: 945- 959

Liu Z H, Wang H Y, Wang X E, Zhang G P, Chen P D, Liu D J, (2006) Genotypic and spike positional difference in grain phytase activity, phytate, inorganic phosphorus, iron, and zinc contents in wheat (*Triticum aestivum* L*). **J Cereal Sci.*** **44**: 212-219

Luo Y, Xie W, Jin X, Zhang B, Wang Q and He Y (2013). The impact of processing on phytic acid, in vitro soluble zinc and Phy/Zn molar ratio of faba bean (*Vicia faba* L) ***Int Food Res J.*** **20(3)**:1285-1291

Messina M J (1999) Legume and soybeans: overview of their nutritional profiles and health effects. ***Am J Clin Nutr.*****70 (3 Suppl**): 439S-449S

Mubarak A E (2005). Nutritional composition and anti-nutritional factors of mungbean seeds (*Phaseolus aureus*) as affected by some home traditional processes. ***Food Chem.*****89**:489-495

Odeny, D A (2007). The potential of pigeonpea (*Cajanus cajan* (L.) Millsp.) in Africa. ***Natural resources forum.*** **31**: 297-305

Peleg Z, Cakmak I, Ozturk L, Yazici A, Jun Y, Budak H, Saranga Y (2009). Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat× wild emmer wheat RIL population. ***Theor Appl Genet.*** **119(2)**: 353-369

Prentice A M, Gershwin M E, Schaible U E, Keusch G T, Victora C G, and Gordon J I. (2008) New challenges in studying nutrition-disease interactions in the developing world. ***J Clin Invest.*** **118(4)**: 1322-1329

Rocio Campos-Vega, Guadalupe Loarca-Pina, Dave Oomah (2010) Minor components of pulses and their potential impact on human health. ***Food Res Int.*****43**:461-482

Sandberg AS and Svanberg U (1991). Phytate hydrolysis by phytase in cereals. Effects on in vitro estimation of iron availability. ***J Food Sci.*** **56**: 1330-1333

Saroj Kumar Prasad & Manoj Kumar Singh (2014) Horse gram- an underutilized nutraceutical pulse crop***. J Food Sci Technol.*** **52(5)**: 2489-2499

Shamsuddin AKM and Vucenik I (2005) IP6 and inositol in cancer prevention and therapy. ***Curr Canc Ther Rev.*****1**: 259-269

Shi R L, Li H W, Tong Y P, Jing R L, Zhang F S, Zou C Q (2008) Identification of quantitative trait locus of zinc and phosphorus density in wheat (*Triticum aestivum* L) grain. ***Plant Soil.*** **306**: 95-104

Siddhuraju P and Becker K (2007) The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* L. Walp.) seed extracts. ***Food Chem.*** **101**:10-19

Stewart C P, Dewey K G and Ashorn P (2010) The undernutrition epidemic: an urgent health priority. ***The Lancet.*** **375(9711)**: 282.

Torres A, Frias J, Granito M and Vidal-Valverde C (2006) Fermented Pigeon Pea (*Cajanus cajan*) ingredients in Paste Products. ***J Agric Food Chem.*** **54(18)**:6685-6691

Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J(2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. ***Pub Med Science.*** **314**: 1298-1301

Vardis Dilis**,** Antonia Trichopoulou (2009) Nutritional and health properties of pulses. ***Mediterr J Nutr Metab.*** **1**:149-157

Venn BJ and Mann JI (2004) Cereal grains, legumes and diabetes**.** ***Eur J Clin Nutr.*** **58**:1443-1461

Welch R. M., and Graham R D (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. ***J Exp Bot.*** **55(396)**:353-364

White P J, and Broadley M R (2009) Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. ***New Phytologist.*** **182(1)**: 49-84

Xu Y, An D, Li H, and Xu, H (2011) Breeding wheat for enhanced micronutrients. ***Can J Plant Sci.*** **91**: 231-237

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